

Attorney Docket No.: T5530.CIP  
Inventors: Rao et al.  
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comprising the steps of:

(a) isolating a population of [mammalian] rodent or human multipotent CNS stem cells [capable of generating] which generate both neurons and glia;

(b) incubating the multipotent CNS stem cells in [a] NEP medium configured for inducing said cells to begin differentiating;

(c) purifying from the differentiating cells a subpopulation of cells expressing [a selected antigen defining neuron-restricted precursor cells] embryonic neural cell adhesion molecule via a procedure selected from the group consisting of specific antibody capture, fluorescence activated cell sorting, and magnetic bead capture; and

(d) incubating the purified subpopulation of cells in a medium configured for supporting adherent growth thereof to obtain an isolated, purified population of rodent or human CNS neuron-restricted precursor cells, wherein said neuron-restricted precursor cells require FGF and differentiate into CNS neuronal cells but not into CNS glial cells.

In claim 15, please delete "14" and insert --12--.

21. (amended) A method of isolating a pure population of

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[mammalian] rodent or human CNS neuron-restricted precursor cells comprising the steps of:

- (a) removing a sample of [CNS] spinal cord tissue from a [mammalian] rodent or human embryo at a stage of embryonic development after closure of the neural tube but prior to differentiation of glial and neuronal cells in the neural tube;
- (b) dissociating cells comprising the sample of [CNS] spinal cord tissue removed from the [mammalian] embryo;
- (c) purifying from the dissociated cells a subpopulation expressing [a selected antigen defining neuron-restricted precursor cells] embryonic neural cell adhesion molecule;
- (d) plating the purified subpopulation of cells in feeder-cell-independent culture on a substratum and in a medium configured for supporting adherent growth of the neuron-restricted precursor cells; and
- (e) incubating the plated cells at a temperature and in an atmosphere conducive to growth [of the] to obtain an isolated, pure population of neuron-restricted precursor cells, wherein said neuron-restricted precursor cells require FGF and differentiate into CNS neuronal cells but not into CNS glial cells.

26. (amended) A pure population of [mammalian] rodent or human

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*but G*  
CNS neuron-restricted precursor cells isolated by the method of claim 12, wherein said neuron-restricted precursor cells require FGF and differentiate into CNS neuronal cells but not into CNS glial cells.

*R*  
27. (amended) A pure population of [mammalian] rodent or human CNS neuron-restricted precursor cells isolated by the method of claim 21, wherein said neuron-restricted precursor cells require FGF and differentiate into CNS neuronal cells but not into CNS glial cells.

*S*  
28. (amended) A method of [obtaining] producing postmitotic neurons from neuron-restricted precursor cells comprising:  
(a) [providing neuron-restricted precursor cells and] culturing [the] neuron-restricted precursor cells which require FGF and differentiate into CNS neuronal cells but not into CNS glial cells in proliferating conditions; and  
(b) changing the culture conditions of the neuron-restricted precursor cells from proliferating conditions to differentiating conditions, thereby causing the neuron-restricted precursor cells to differentiate into postmitotic neurons.

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*A 4*  
35. (amended) A pharmaceutical composition comprising a therapeutically effective amount of a composition [of Claim 34] comprising rodent or human CNS neuron-restricted precursor cells which require FGF and differentiate into CNS neuronal cells but not into CNS glial cells and a pharmaceutically acceptable carrier.

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*A 5*  
37. (amended) A method for treating a neuronal disorder in a mammal comprising [administering to] transplanting into said mammal at or near any regions of the central nervous system affected by the neuronal disorder a therapeutically effective amount of the pharmaceutical composition of Claim 35 wherein said neuronal disorder comprises Parkinson's disease, Huntington's disease, Alzheimer's disease, dysfunctions resulting from injury or trauma, amyotrophic lateral sclerosis, or anencephaly.

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*A 4*  
43. (amended) [A] The method [for treating neurodegenerative symptoms in a mammal comprising the steps of:  
    (a) providing a pure population of neuronal restricted precursor cells;  
    (b)] of claim 45 wherein said transplanted neuron-restricted precursor cells are genetically [transforming said neuronal restricted precursor cells] transformed with a gene

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encoding a growth factor, neurotransmitter, neurotransmitter synthesizing enzyme, neuropeptide, neuropeptide synthesizing enzyme, or substance that provides protection against free-radical mediated damage thereby resulting in a transformed population of [neuronal] neuron-restricted precursor cells that express said growth factor, neurotransmitter, neurotransmitter synthesizing enzyme, neuropeptide, neuropeptide synthesizing enzyme, or substance that provides protection against free-radical mediated damage[; and

(c) administering an effective amount of said transformed population of neuronal restricted precursor cells to said mammal].

44. (amended) A method [or] for screening compounds for neurological activity comprising the steps of:

(a) providing a pure population of [neuronal] neuron-restricted precursor cells or derivatives thereof or mixtures thereof cultured in vitro, wherein said cells or derivatives thereof or mixtures thereof require FGF and differentiate into CNS neuronal cells but not into CNS glial cells;

(b) exposing said cells or derivatives thereof or mixtures thereof to a selected compound at varying dosages; and

(c) monitoring the reaction of said cells or derivatives

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thereof or mixtures thereof to ~~said~~ selected compound for selected time periods.

45. (amended) A method for treating a neurological or neurodegenerative disease comprising [administering to] transplanting into a mammal in need of such treatment an effective amount of [neuronal] neuron-restricted precursor cells or derivatives thereof or mixtures thereof which require FGF and differentiate into CNS neuronal cells but not into CNS glial cells at or near any regions of the central nervous system affected by the neurological or neurodegenerative disease wherein said neurological or neurodegenerative disease comprises Parkinson's disease, Huntington's disease, Alzheimer's disease, dysfunctions resulting from injury or trauma, amyotrophic lateral sclerosis, or anencephaly.

59. (amended) A method of isolating a pure population of [mammalian] mouse or human CNS neuron-restricted precursor cells [comprises] comprising the steps of:  
(a) providing a sample of [mammalian] mouse or human embryonic stem cells;  
(b) purifying from the [mammalian] mouse or human embryonic

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stem cells a subpopulation expressing [a selected antigen defining neuron-restricted precursor cells] embryonic neural cell adhesion molecule;

(c) plating the purified subpopulation of cells in feeder-cell-independent culture on a substratum and in a medium configured for supporting adherent growth of the neuron-restricted precursor cells; and

(d) incubating the plated cells at a temperature and in an atmosphere conducive to growth of the neuron-restricted precursor cells, wherein said neuron-restricted precursor cells require FGF and differentiate into CNS neuronal cells but not into CNS glial cells.

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Please cancel claims 1-11, 13, 14, 17, 18, 19, 20, 22, 25, 34, 36, 38, 39, 40, 41, 42, 46, 47 and 48.

#### REMARKS

Claims 1-59 are pending in the instant application. Claims 1-59 have been rejected. Claims 12, 15, 21, 26, 27, 28, 35, 37, 43, 44, 45 and 59 have been amended. Claims 1-11, 13, 14, 17, 18, 19, 20, 22, 25, 34, 36, 38, 39, 40, 41, 42, 46, 47 and 48 have been canceled. No new matter has been added by these amendments.